Front. Earth Sci. China 2010, 4(2): 160–166
DOI 10.1007/s11707-010-0020-x

RESEARCH ARTICLE

Goethite-enhanced anaerobic bio-decomposition of sulfate minerals

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Abstract The effects of goethite on the anaerobic bio-decomposition of sulfate minerals were investigated by using the beef extract as a carbon source and the activated sludge as the source of mixed bacteria. Solution pH, sulfate concentration, total iron ion concentration and the solid products of four different batch tests were monitored and analyzed. Experimental results showed that the presence of iron oxide can improve the alkalinity of the reaction system which results in the increase of pH value. Moreover, the added goethite consumed a large amount of H_2S generated from the sulfate minerals by SRB, thereby significantly enhancing the decomposition of gypsum and anhydrite compared with the control batch tests. In addition, the SEM observation and the EDS spectra showed that there were some rod-shaped microorganisms and new generated minerals, such as iron sulfide, calcium carbonate crystals, and elemental sulfur. Both of the proliferation of new minerals (iron sulfide and calcium carbonate crystals) and the complete disappearance of sulfate minerals indicated that iron oxide can play the role in fixing the S element through the metabolism of SRB and hence improve the bio-decomposition of sulfate minerals.

Keywords goethite, sulfate-reducing bacteria, sulfate minerals, gypsum, anhydrite

1 Introduction

Sulfate-reducing bacteria (SRB) are either heterotrophic or autotrophic anaerobes, capable of reducing sulfate to sulfide by a dissimulator, bioenergetic metabolism when provided with a suitable organic carbon source (Wang et al., 2009). SRB have been successfully used in the treatment of waters and leachates in large-scale bioreactors and in pilot plants (Gadd and White, 1993; Gadd, 2000). Mixed sulfate-reducing bacterial consortia are proved to be effective in the removal of heavy metals from solutions. SRB-containing reactors have also been used in the removal of heavy metals from soils (Neculita et al., 2007). SRB and iron-reducing bacteria such as *Shewanella* and *Geobacter species* were often used for the remediation processes in situ due to their importance in the immobilization of heavy metals in natural sediments (Fredrickson and Gorby, 1996; Labrenz et al., 2000; Valls and de Lorenzo, 2002). However, the buildup of sulfide and its subsequent transfer to the gas phase induce odour and corrosion problems. Additionally, the large number of dissolved sulfide has toxic effects on microorganisms, resulting in weak activity or even death (Wang et al., 2009). Many technologies are being used to minimize the adverse effect of hydrogen sulfide, such as the injection/addition of air, pure oxygen or nitrate salts to prevent sulfide formation (Ochi et al., 1998) and the addition of ozone, hydrogen peroxide, hypochlorites, chlorine, and potassium permanganate to chemically oxidized sulfide (Cadena and Peters, 1988; Millero et al., 1989; Tomar and Abdullah, 1994; Charron et al., 2004). However, the high cost of operation or the complex operations limited the popular use of the above methods.

Therefore, the addition of metal salts, including iron, zinc, lead and copper salts, was used to precipitate sulfide by forming highly insoluble metallic sulfide precipitates (Padival et al., 1995; Poulton et al., 2002; Zhang et al., 2009). Substrate (electron donor) oxidation by SRB is coupled to sulfate (terminal electron acceptor) reduction. Furthermore, the dissolved inorganic carbon neutralizes the pH and favors the precipitation of metal carbonate minerals. The soluble sulfides (H_2S, HS^-, and S^{2-}) react with metals to form metal sulfide precipitates. The reaction is generally expressed as (Neculita et al., 2007):

Received December 7, 2009; accepted January 18, 2010

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$$2CH_2O(\text{organics}) + SO_4^{2-} \xrightarrow{\text{SRB}} H_2S + 2HCO_3^- \quad (1)$$

$$M^{2+} + H_2S + 2HCO_3^- \rightarrow MS + 4H^+ + 2CO_3^{2-} \quad (2)$$

where CH_2O and M respectively represent a simple organic carbon source and a cationic metal such as Cd, Fe, Ni, Cu, or Zn. Fe^{3+} is most widely used and effective in decreasing dissolved sulfide from wastewater (Nielsen et al., 2005; Zhang et al., 2009). Goethite [α-FeOOH] is a kind of source of Fe^{3+}, which has been implicated in the pathways for hydrolysis, reduction and oxidation (Holmén and Casey, 1996). As this phase of iron is the most widespread mineral in natural systems, its effects on environment are important in terms of migration of metals in natural waters and mobility of toxic elements to living organisms (Mustafa et al., 2009).

On the other hand, there are a large number of sulfate minerals in nature. Gypsum [$CaSO_4 \cdot 2H_2O$] and anhydrite [$CaSO_4$] are the two calcium sulfate minerals occurring in nature. Gypsum is the hydrated form of calcium sulfate in which the water and calcium sulfate are intimately bound together. It is the combined water that makes gypsum an important commercial mineral (McGregor, 1954). Fluorine gypsum, phosphogypsum and gypsum are a major source of industry by-product gypsum, which has a serious harm to the environment in terms of the occupation of land, pollution of water and atmosphere, etc. (Yang, 2006). However, the information about the pollution control and treatment for gypsum mining is still limited.

To investigate the effects of iron oxide on the decomposition of sulfate minerals by microorganisms, gypsum and anhydrite were selected as sulfate minerals for the sulfate donor, and goethite was served as the representative of iron oxide in this paper. The performances of four different batch tests were monitored and analyzed with regard to the pH, sulfate concentration, total iron ion concentration and the final product.

2 Materials and methods

2.1 Chemicals

The gypsum and anhydrite were from Dingyuan Gypsum Mine (Anhui Province, China) and Hengtai Co. (Anhui Province, China), respectively. Goethite was from Zhenjiang Fine Chemical Plant. As shown in Fig. 1, the anhydrite and goethite contain dolomite and calcite, respectively; it was pretreated by heating and pickling to remove the impurities.

2.2 Organism, media and cultivation

The SRB-rich activated sludge was inoculated as microbial inoculum. Strict anaerobic techniques were used throughout the gypsum and anhydrite decomposition test. To investigate the effect of goethite on the decomposition of sulfate minerals, four different media and 3 mL sludge supernatant of 6.7 mg COD/L were respectively dispensed into serum bottles (50 mL) for each batch test, as shown below.

Medium 1 (Batch 1): Beef extract 2 g COD/L and gypsum 4 g/L.

Medium 2 (Batch 2): Beef extract 2 g COD/L, gypsum 4 g/L, and goethite 8 g/L.

Medium 3 (Batch 3): Beef extract 2 g COD/L and anhydrite 4 g/L.

Medium 4 (Batch 4): Beef extract 2 g COD/L, anhydrite 4 g/L, and goethite 8 g/L.

The bottles were purged with O_2-free N_2/CO_2 (80/20 v/v) for 15 min, sealed with butyl-rubber stoppers and crimped with aluminum caps. All bottles were incubated in the dark at 30°C, which was optimal for the microbial growth of SRB. Intermittent sampling was conducted during the reaction for the analysis of pH values, SO_4^{2-} concentration, COD and total iron concentration. After reaction, the precipitates were collected to characterize by

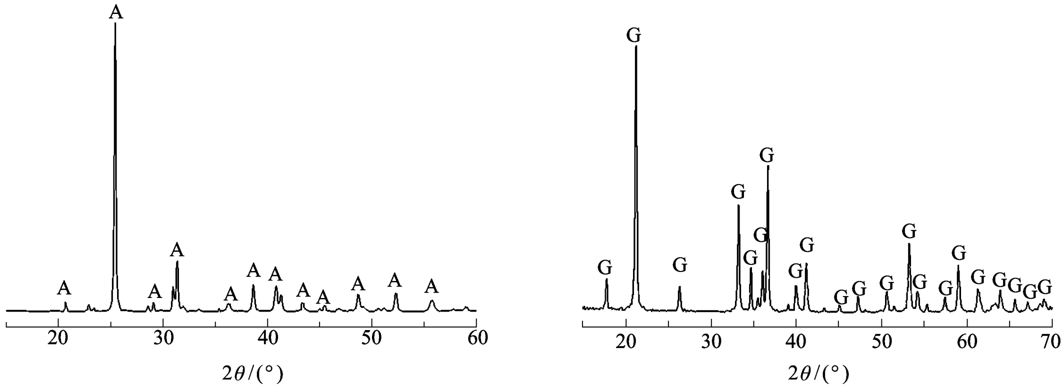


Fig. 1 X-ray diffraction pattern of goethite and anhydrite sample after pretreatment with acid (A-anhydrite; G-goethite)

the scanning electron microscope (SEM), energy dispersive spectrum (EDS) and X-ray diffraction (XRD).

2.3 Analysis

The SO_4^{2-} concentration and iron ion concentration were determined using spectrophotometry method (APHA, 1989). After completion of the reaction, the samples were centrifugated to get the black precipitates. The precipitates were washed by anhydrous alcohol and centrifugated for 3 times and used for the SEM and EDS analysis (Sirion200, FEI, USA). The SEM and EDS analysis was conducted in Hefei National Laboratory for Physical Science at the Microscale (China). The XRD analysis of anhydrite and goethite was conducted using the D/MAX2500VL/PC X-ray diffraction instrument (Japan).

3 Results and discussion

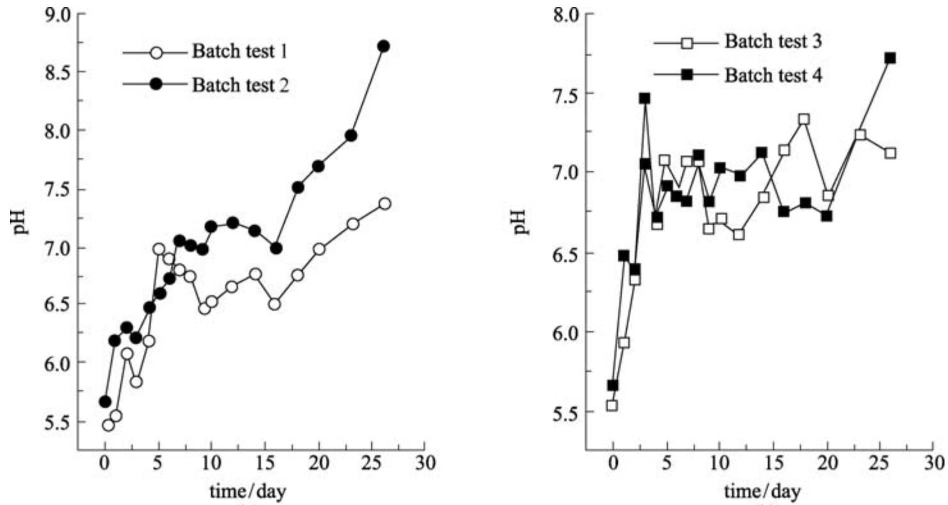
3.1 Effects of iron oxide on pH

At the end of the reaction, the pH value of each different test was raised from 5.5 respectively to 7.36, 8.71, 7.13 and 7.75. As shown in Fig. 2, the pH values of Batch 2 and Batch 4 were slightly higher than the pH values of the corresponding control batch test without iron oxide addition. This indicates that the presence of iron oxide can improve the alkalinity of the reaction system which results in the increase of the pH value. Moreover, it was found during the biotreatment of zinc-containing wastewater that alkalinity produced from acetate oxidation increased the wastewater pH remarkably (Sahinkaya, 2009). The effects of hydraulic retention time (HRT) and sulfide toxicity on ethanol and acetate utilization were studied in a sulfate-reducing fluidized-bed reactor (FBR) treating acidic metal-containing wastewater by Kaksonen et al. (2006). They also found that the alkalinity produced by substrate utilization increased the wastewater pH from 3 to 7.9–8.0. According to Eq. (1), this may be due to the fact that sulfate minerals have been reduced to hydrogen sulfide by the SRB, and at the same time, the HCO_3^- produced can increase the alkalinity. Moreover, the addition of iron oxide may lead to the following reaction, which consumes H^+ to increase pH values and promote the dissolution of CO_2, hereby to form calcium carbonate crystals (Neal et al., 2001).

$$Fe_2O_3/FeOOH + 6H^+ + 2e^- \rightarrow 2Fe^{2+} + 3H_2O \qquad (3)$$

3.2 Effects of iron oxide on SO_4^{2-} concentration

Figure 3 shows that the sulfate concentrations in the four batch tests all presented an increase first followed by a decrease. The initial increase of the sulfate concentration was caused by the decomposition of the sulfate minerals, while the subsequent decrease of the sulfate concentration was contributed to the sulfate consumption by the SRB according to Eq. (1). Furthermore, the sulfate concentration in Batch 2 and Batch 4 with iron oxide addition reached its maximum around the fifth day, while the sulfate concentration in Batch 1 and Batch 3 without iron oxide addition reached its maximum on day 7. In addition, the sulfate concentration in Batch 2 and Batch 4 was much lower than its value in the corresponding control batch test. This indicated that the iron oxide added can consume the H_2S produced to enhance the decomposition of the sulfate minerals, according to Eqs. (1) and (4).



(a) (b)

Fig. 2 Variation of the pH values of culture media respectively using (a) gypsum and (b) anhydrite as sulfate minerals with (Batch 2 and Batch 4) or without (Batch 1 and Batch 3) goethite addition

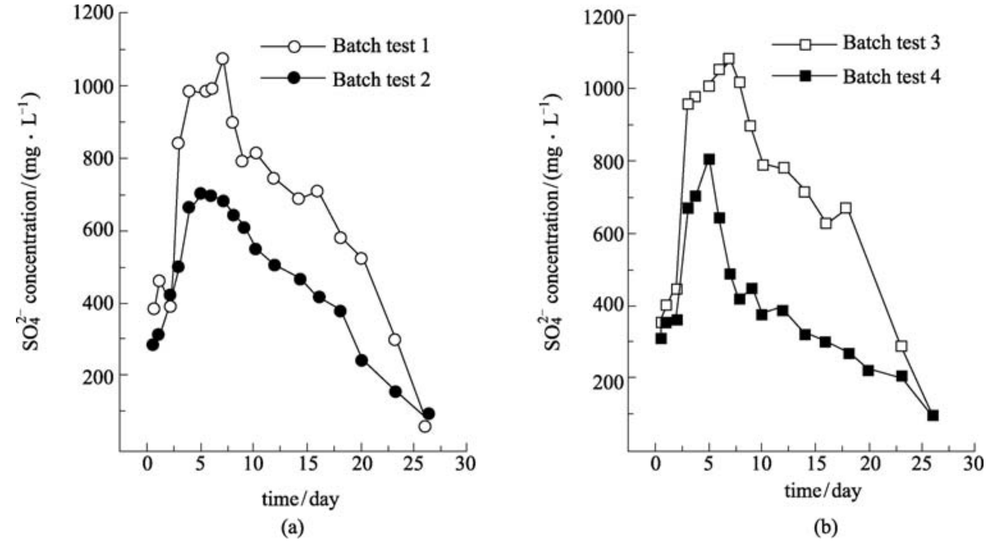


Fig. 3 Variation of the sulfate concentration in culture media respectively using (a) gypsum and (b) anhydrite as sulfate minerals with (Batch 2 and Batch 4) or without (Batch 1 and Batch 3) goethite addition

$$2FeOOH + H_2S + 4H^+ \rightarrow 2Fe^{2+} + S^0 + 4H_2O \qquad (4)$$

Gypsum and anhydrite are typical sulfate minerals. They showed slow-release characteristics as the sulfate donor of the sulfate reduction reaction. With the reaction, gypsum or anhydrite gradually dissolved into the aqueous environment, and sulfate concentrations gradually increased. In this study, sulfate-reducing bacteria consumed beef extracts as the carbon and energy sources for its growth and metabolism. Sulfate minerals provided sulfate as the electron acceptor. The metabolic process for sulfate-reducing bacteria is divided into three steps, i.e., catabolism, electron transfer and oxidation (Utgikar et al., 2002). The main metabolic process during the catabolism is the anaerobic degradation of organic carbon and the production of a small amount of ATP by substrate-level phosphorylation reaction. Subsequently, in the electron transfer stage, the energetic electrons previously produced were progressively transferred by the inherent electron transport chain of sulfate-reducing bacteria and produce a large number of ATPs. Finally, a certain amount of ATP was consumed to promote the reduction of sulfur in oxidation state to S^{2-} or sulfur (Hu et al., 2003).

3.3 Variation of total iron ion concentration

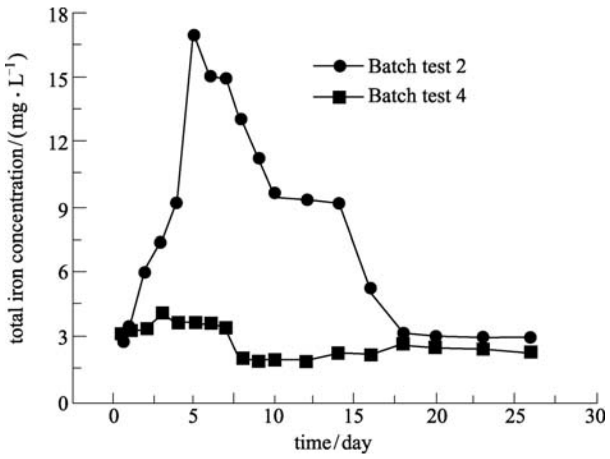


Fig. 4 Variation of the total iron ion concentration in culture media respectively using gypsum (Batch test 2) and anhydrite (Batch test 4) as sulfate minerals

As shown in Fig. 4, the total iron ion concentration in Batch 2 initially increased from 2 mg/L to 17 mg/L and then gradually decreased to around 3 mg/L. However, the total iron ion concentration in Batch 4 only increased slightly in the initial cultivation and soon dropped to around 1.7 mg/L. The variation of total iron ion concentration may be due to the following reactions which may occur in this study (Hu et al., 2003):

$$2CH_2O(\text{organics}) + CaSO_4 \xrightarrow{SRB} H_2S + 2HCO_3^- + Ca^{2+} \qquad (5)$$

$$Fe^{3+} \rightarrow Fe^{2+} \qquad (6)$$

$$Fe^{2+} + S^{2-} \rightarrow FeS \qquad (7)$$

$$HS^- \rightarrow H^+ + S^{2-} \tag{8}$$

$$H_2S(\text{aqueous}) \rightarrow HS^- + H^+ \tag{9}$$

$$3H_2S + 2Fe^{3+} \rightarrow S^0 + 2FeS + 6H^+ \tag{10}$$

$$Fe^{2+} + H_2S + 2Ca^{2+} + 2HCO_3^- + 4e^- \rightarrow FeS + 2CaCO_3 \tag{11}$$

The accumulation of iron ion in the first several days was mainly contributed to the decomposition of the goethite and the reduction of Fe^{3+}. The reduction reaction of Fe^{3+} is a key issue for the above series reactions. Fe^{3+} is often the dominant electron-accepting process in acidic sediments, and the reduction of sulfate occurs in sediments with elevated pH values. Moreover, Fe^{3+} can be reduced by some iron-reducing bacteria (Kusel et al., 2001). Since the activated sludge was used as the bacteria inoculum in this experiment, the bacterial species were rich and might contain a number of iron-reducing bacteria. This can be proved by the phenomena of Fe^{3+} reduction to some extent. In the anaerobic system, iron ion concentration in the solution increased gradually with the role of iron-reducing bacteria. The subsequent decrease of iron ion concentration was due to the consumption of Fe^{2+} by generating iron sulfide, according to Eqs. (7), (10) and (11). Beef extract is a kind of popular carbon sources for bacteria under anaerobic conditions. It can be fermented to produce low molecular product (i.e. acetic acid), hydrogen, carbon dioxide and some other gases (Hu et al., 2003). It was proved in our previous study that hydrogen was not able to reduce Fe^{3+} to Fe^{2+} at room temperature while it did work at high-temperature conditions (Liu et al., 2010). As shown in Eq. (10), hydrogen sulfide reduces Fe^{3+} first and followed by FeS precipitation due to the immediate reaction of the produced Fe^{2+} with S^{2-} . On the other hand, the produced Fe^{2+} also can react with hydrogen sulfide and calcium ion to form FeS precipitation and calcium carbonate crystals with the supply of alkalinity and electron, as shown in Eq. (11).

The generation and consumption of sulfide can be well explained by Eqs. (1)–(11). As the sulfate concentration increased due to the decomposition of sulfate minerals by SRB (Fig. 3), sulfate reduction started to occur, accompanied by a large number of hydrogen sulfide gases. As shown in Eqs. (8) and (9), part of the hydrogen sulfide gas dissolved into the solution under the ionization equilibrium. Furthermore, the desulfurization reaction can occur directly by reducing Fe^{3+} to be iron sulfide and sulfur with another part of the produced hydrogen sulfide gas (Eq. (10)). Thus, the sulfide concentration in the solution decreased, which would degrade the toxic effects of sulfide on the microorganisms. Moreover, as shown in Eqs. (6), (7), (10) and (11), the consumption of sulfide would diminish the inhibition of hydrogen sulfide on sulfate reduction and improve the release and consumption of iron ion from goethite. As shown in Fig. 4, total iron ion concentration was much lower in Batch test 4 than in Batch test 2. This might be due to the higher sulfate content in anhydrite than in gypsum with the same initial weight. More sulfates were reduced to generate H_2S to precipitate more iron ion in Batch test 4 than in Batch test 2, which led to a lower accumulation of total iron ion. Therefore, the addition of goethite can enhance the bio-decomposition of both gypsum and anhydrite.

3.4 SEM, EDS and XRD analysis

Figure 5 shows the SEM images and EDS spectrum for the products of the bio-decomposition process of sulfate minerals. There were some rod-shaped microorganisms (Fig. 5 (a) label B), a large number of petal-shaped iron sulfide minerals (Fig. 5(a) label P), some rhomb-shaped calcium carbonate crystals (Fig. 5(a) label C), and a very little amount of elemental sulfur (Fig. 5(b) label S).

As shown in Fig. 6, the precipitates from batch tests were also analyzed by XRD. The results showed that gypsum was decomposed completely to produce calcium carbonate crystals, and there is still some amount of goethite left. The XRD analysis further proved that goethite can improve the bio-decomposition of sulfate minerals.

The proliferation of iron sulfide and the complete disappearance of sulfate minerals indicated that iron oxide can play the role in fixing S element through the metabolism of SRB and hence improve the bio-decomposition of sulfate minerals. The generation of calcium carbonate crystals further confirmed the reaction described by Eq. (11), which avoided the accumulation of calcium ions in the solution and improved the bio-decomposition of sulfate minerals from another point of view.

4 Conclusions

In this paper, the effects of goethite on the anaerobic bio-decomposition of sulfate minerals were explored, and the following results are drawn:

1) The presence of goethite can improve the alkalinity of the reaction system which results in the increase of the pH value.

2) Goethite can enhance the decomposition of gypsum and anhydrite by reacting with H_2S generated from the sulfate minerals by SRB to form iron sulfide.

3) There were some rod-shaped microorganisms and new generated minerals, such as iron sulfide, calcium carbonate crystals, and elemental sulfur in the precipitants after the end of the anaerobic cultivation.

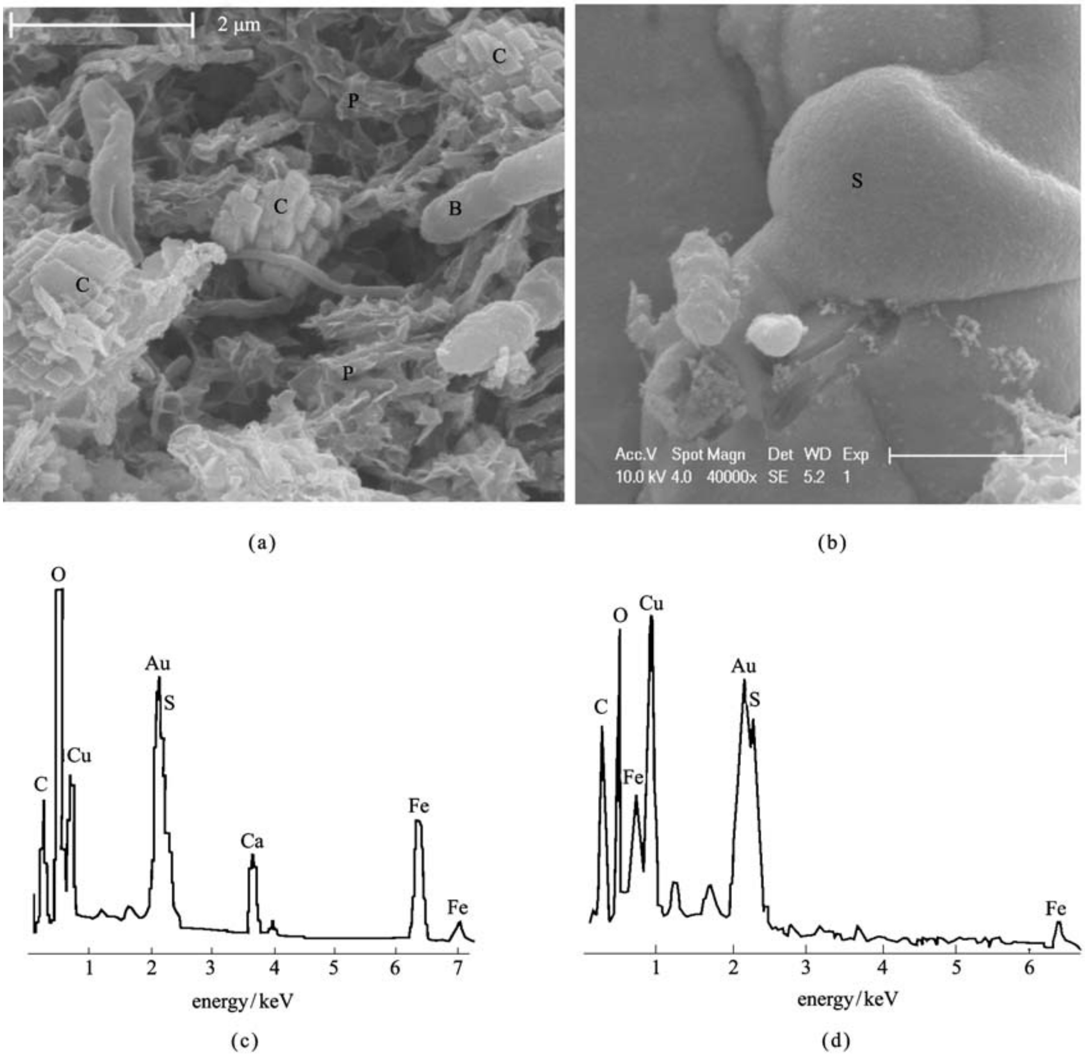


Fig. 5 SEM images (a, b) and EDS spectrum (c, d) of the precipitates from batch tests. C, B, P and S represent calcite, bacteria, iron sulfide and elemental sulfur, respectively

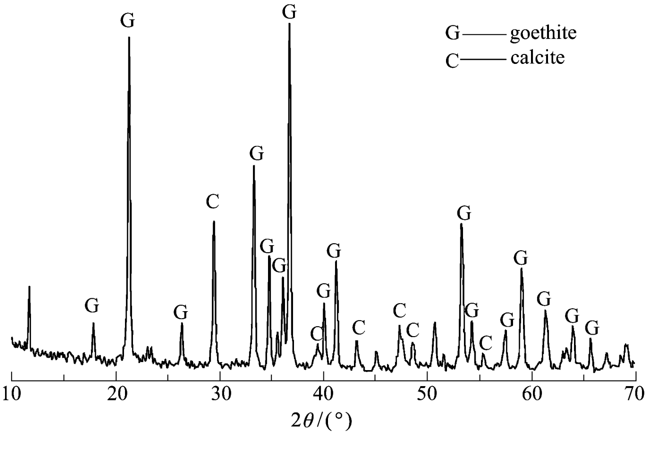


Fig. 6 XRD spectrum of the precipitates from batch tests

Acknowledgements This study was supported by the National Natural Science Foundation of China (Grant Nos. 40902019 and 40872047) and the National Basic Research Program of China (2007CB815603).

References

APHA (1989). Standard Methods for the Examination of Water and Wastewater. 17th ed. Washington D C: American Public Health Association

Cadena F, Peters R W (1988). Evaluation of chemical oxidizers for hydrogen sulfide control. J Water Pollut Control Fed, 60: 1259–1263

Charron I, Féliers C, Couvert A, Laplanche A, Patria L, Requieme B (2004). Use of hydrogen peroxide in scrubbing towers for odor removal in wastewater treatment plants. Water Sci Technol, 50(4): 267–274

Fredrickson J K, Gorby Y A (1996). Environmental processes mediated by iron-reducing bacteria. Curr Opin Biotechnol, 7 (3): 287–294

Gadd G M, White C (1993). Microbial treatment of metal pollution—a working biotechnology? Trends Biotechnol, 11 (8): 353–359

Gadd G M (2000). Accumulation and Transformation of Metals by Microorganisms. New York: John Wiley and Sons Inc

Holmén B A, Casey W H (1996). Hydroxamate ligands, surface chemistry, and the mechanism of ligand-promoted dissolution of goethite [α-FeOOH(s)]. Geochim Cosmochim Acta, 60 (22): 4403–4416

Hu J C, Zhou M J, Zuo J E (2003). Anaerobic Biological Treatment Theory and Technology. Beijing: China Construction Industry Publishing

Kaksonen A H, Plumb J J, Robertson W J, Riekkola-Vanhanenc M, Franzmannb P D, Puhakkaa J A (2006). The performance, kinetics and microbiology of sulfidogenic fluidized-bed treatment of acidic metal- and sulfate-containing wastewater. Hydrometallurgy, 83(1–4) SI: 204–213

Küsel K, Roth U, Trinkwalter T, Peiffer S (2001). Effect of pH on the anaerobic microbial cycling of sulfur in mining-impacted freshwater lake sediments. Environ Exp Bot, 46 (3): 213–223

Labrenz M, Druschel G K, Thomsen-Ebert T, Gilbert B, Welch S A, Kemner K M, Logan G A, Summons R E, De Stasio G, Bond P L, Lai B, Kelly S D, Banfield J F (2000). Formation of sphalerite (ZnS) deposits in natural biofilms of sulfate-reducing bacteria. Science, 290 (5497): 1744–1747

Liu Y, Chen T H, Sun Y B (2010). Goethite reduction by hydrogen for the removal of nitrate from water. China Water & Wastewater (In press)

McGregor D J (1954). Gypsum and anhydrite deposits in southwestern Indianan. Indiana Geological Survey Report of Progress, 8

Millero F J, Leferriere A, Fernandez M, Hubinger S, Hershey J P (1989). Oxidation of H_2S with H_2O_2 in natural waters. Environ Sci Technol, 23: 209–213

Mustafa S, Khan S, Iqbal Zaman M (2010). Effect of Ni($^{2+}$) ion doping on the physical characteristics and chromate adsorption behavior of goethite. Water Res, 44 (3): 918–926

Neculita C M, Zagury G J, Bussière B (2007). Passive treatment of acid mine drainage in bioreactors using sulfate-reducing bacteria: critical review and research needs. J Environ Qual, 36 (1): 1–16

Nielsen A H, Lens P, Vollertsen J, Jacobsen T H (2005). Sulfide-iron interactions in domestic wastewater from a gravity sewer. Water Res, 39 (12): 2747–2755

Neal A L, Techkarnjanaruk S, Dohnalkova A, McCready D, Peyton B M, Geesey G G (2001). Iron sulfides and sulfur species produced at hematite surfaces in the presence of sulfate-reducing bacteria. Geochim Cosmochim Acta, 65 (2): 223–235

Ochi T, Kitagawa M, Tanaka S. Controlling sulfide generation in force mains by air injection. Water Sci Technol, 1998, 37 (1): 87–95

Padival N A, Kimbell W A, Redner J A (1995). Use of iron salts to control dissolved sulfide in trunk sewers. J Environ Eng, 121 (11): 824–829

Poulton S W, Krom M D, van Rijn J, Raiswell R (2002). The use of hydrous iron (III) oxides for the removal of hydrogen sulphide in aqueous systems. Water Res, 36 (4): 825–834

Sahinkaya E (2009). Biotreatment of zinc-containing wastewater in a sulfidogenic CSTR: Performance and artificial neural network (ANN) modelling studies. J Hazard Mater, 164 (1): 105–113

Tomar M, Abdullah T H A (1994). Evaluation of chemicals to control the generation of malodorous hydrogen sulfide in waste water. Water Res, 28 (12): 2545–2552

Utgikar V P, Harmon S M, Chaudhary N, Tabak H H, Govind R, Haines J R (2002). Inhibition of sulfate-reducing bacteria by metal sulfide formation in bioremediation of acid mine drainage. Environ Toxicol, 17 (1): 40–48

Valls M, de Lorenzo V (2002). Exploiting the genetic and biochemical capacities of bacteria for the remediation of heavy metal pollution. FEMS Microbiol Rev, 26 (4): 327–338

Wang J, Lu H, Chen G H, Lau G N, Tsang W L, van Loosdrecht MCM (2009). A novel sulfate reduction, autotrophic denitrification, nitrification integrated (SANI) process for saline wastewater treatment. Water Res, 43 (9): 2363–2372

Yang X Y (2006). Actuality and resourceful application of gypsum tailings. China Mining Magazine, 15 (4): 37–38 (in Chinese)

Zhang L S, Keller J, Yuan Z G (2009). Inhibition of sulfate-reducing and methanogenic activities of anaerobic sewer biofilms by ferric iron dosing. Water Res, 43 (17): 4123–4132